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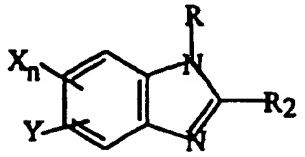
(57) Abstract

A pharmaceutical composition for the treatment of leukemia in mammals is disclosed. The particular fungicide used is a benzimidazole derivative of formula (I) wherein X is hydrogen, halogen, alkyl of less than 7 carbon atoms or alkoxy of less than 7 carbon atoms; n is a positive integer of less than 4; Y is hydrogen, chlorine, nitro, methyl or ethyl; and R is hydrogen or an alkyl group having from 1 to 8 carbon atoms, and R_2 is 4-thiazolyl or $NHCOOR_1$ wherein R_1 is aliphatic hydrocarbon of less than 7 carbon atoms or the pharmaceutically acceptable inorganic or acid addition salts thereof. A pharmaceutical composition that inhibits the growth of tumors and cancers in mammals and can be used to treat viral infections that comprises a fungicide in combination with chemotherapeutic agents is disclosed. The particular fungicide used is a benzimidazole derivative. Potentiators can also be included in the composition.



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A PHARMACEUTICAL COMPOSITION CONTAINING BENZIMIDAZOLE FOR INHIBITING THE GROWTH OF CANCERS

TECHNICAL FIELD

This invention is a pharmaceutical composition that inhibits the growth of cancers and tumors, including leukemia, in mammals, particularly in human and warm blooded animals. It is also effective against viruses and can be used to treat viral infections. The composition contains a benzimidazole derivative.

BACKGROUND OF THE INVENTION

Cancers are the leading cause of death in animals and humans. The exact cause of cancer is not known, but links between certain activities such as smoking or exposure to carcinogens and the incidence of certain types of cancers and tumors has been shown by a number of researchers.

Many types of chemotherapeutic agents have been shown to be effective against cancers and tumor cells, but not all types of cancers and tumors respond to these agents. Unfortunately, many of these agents also destroy normal cells. The exact mechanism for the action of these chemotherapeutic agents are not always known.

Despite advances in the field of cancer treatment the leading therapies to date are surgery, radiation, chemotherapy and bone marrow transplants. Chemotherapeutic approaches are said to fight cancers that are metastasized or ones that are particularly aggressive. Such cytocidal or cytostatic agents work best on cancers with large growth factors, i.e., ones whose cells are rapidly dividing. To date, hormones, in particular estrogen, progesterone and testosterone, and some antibiotics produced by a variety of microbes, alkylating agents, and anti-metabolites form the bulk of therapies available to oncologists. Ideally cytotoxic agents that have specificity for cancer and tumor cells while not affecting normal cells would be extremely desirable. Unfortunately, none have been found and instead agents which target especially rapidly dividing cells (both tumor and normal) have been used.

Clearly, the development of materials that would target tumor cells due to some unique specificity for them would be a breakthrough. Alternatively, materials that were cytotoxic to tumor cells while exerting mild effects on normal cells would be desirable. Therefore, it is an object of this invention to provide a pharmaceutical composition that is effective in inhibiting the growth of tumors and cancers in mammals with mild or no effects on normal cells.

More specifically, it is an object of this invention to provide an anti-cancer composition comprising a pharmaceutical carrier and a benzimidazole derivative as defined herein along with a method for treating such cancers.

The development of materials that would target leukemia cells due to some unique specificity for them would also be a breakthrough. Alternatively, materials that were cytotoxic to leukemia cells while exerting mild effects on normal cells would be desirable. Therefore, it is an object of this invention to provide a pharmaceutical composition that is effective in treating leukemia with mild or no effects on normal blood cells

More specifically, it is an object of this invention to provide a composition comprising a pharmaceutical carrier and a benzimidazole derivative as defined herein along with a method for treating leukemia.

It is believed that these benzimidazole compositions when used in conjunction with chemotherapeutic agents can reduce the growth of cancers and tumors, including leukemia. It has been found that the benzimidazoles are especially effective in suppressing the growth of the cancer, tumor, virus, or bacteria. The use of these benzimidazoles in combination with other chemotherapeutic agents which are effective in destroying the tumor is a novel method of treatment.

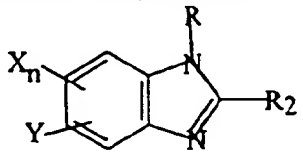
More specifically, it is an object of this invention to provide an anti-cancer composition comprising a pharmaceutical carrier and a benzimidazole derivative and a chemotherapeutic agent as defined herein along with a method for treating such cancers. A potentiator can be used in these compositions.

The benzimidazole compositions are also effective against viruses and can be used to treat viral infections. Therefore it is another object of this invention to provide a method of treating viral infections such as HIV, influenza and rhinoviruses wherein the benzimidazole is administered in conjunction with a potentiator.

These and other objects will become evident from the following detailed description of this inventions.

SUMMARY OF THE INVENTION

A pharmaceutical composition for treatment of cancers, tumors, including leukemia in mammals, and in particular, warm blooded animals and humans, comprising a pharmaceutical carrier and an effective amount anti-cancer compound selected from the group consisting of:



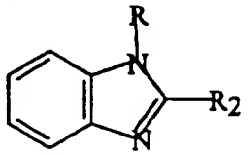
wherein X is hydrogen, halogen, alkyl of less than 7 carbon atoms or alkoxy of less than 7 carbon atoms; n is a positive integer of less than 4; Y is hydrogen, chlorine, nitro, methyl or ethyl; and R is hydrogen, or an alkyl group of from 1 to 8 carbon atoms and R_2 is 4-thiazolyl, $NHCOOR_1$ wherein R_1 is aliphatic hydrocarbon of less than 7 carbon atoms, and preferably an alkyl group of less than 7 carbon atoms is claimed.

These compositions can be used to inhibit the growth of cancers and other tumors in humans or animals by administration of an effective amount either orally, rectally, topically or parenterally, intravenously or by injection into the tumor. These compositions do not significantly affect healthy cells as compared to adriamycin which has a detrimental effect on healthy cells.

A pharmaceutical composition for treatment of mammals, and in particular, warm blooded animals and humans, comprising a pharmaceutical carrier and an effective amount of chemotherapeutic agents and a benzimidazole as described above.

Potentiators can also be used with this composition.

Preferably the compositions are:



wherein R is an alkyl of 1 through 8 carbon atoms and R_2 is selected from the group consisting of 4-thiazolyl, $NHCOOR_1$, wherein R_1 is methyl, ethyl or isopropyl and the non-toxic, pharmaceutically acceptable acid addition salts with both organic and inorganic acids. The most preferred compounds are 2-(4-thiazolyl)benzimidazole, methyl -(butylcarbamoyl)-2-benzimidazolecarbamate and 2-methoxycarbonylamino-benzimidazole and those wherein Y is chloro.

DETAILED DESCRIPTION OF THE INVENTION

A. DEFINITIONS:

As used herein, the term "comprising" means various components can be conjointly employed in the pharmaceutical composition of this invention. Accordingly, the terms "consisting essentially of" and "consisting of" are embodied in the term comprising.

As used herein, a "pharmaceutically acceptable" component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

As used herein, the term "safe and effective amount" refers to the quantity of a component which is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. The specific "safe and effective amount" will, obviously,

vary with such factors as the particular condition being treated, the physical conditi n of the patient, the type of mammal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or its derivatives.

As used herein, a "pharmaceutical addition salts" is salt of the anti-cancer compound with an organic or inorganic acid. These preferred acid addition salts are chlorides, bromides, sulfates, nitrates, phosphates, sulfonates, formates, tartrates, maleates, malates, citrates, benzoates, salicylates, ascorbates, and the like.

As used herein, a "pharmaceutical carrier" is a pharmaceutically acceptable solvent, suspending agent or vehicle, including liposomes, for delivering the anti-cancer agent to the animal or human. The carrier may be liquid or solid and is selected with the planned manner of administration in mind.

As used herein, "cancer" refers to all types of cancers or neoplasm or malignant tumors found in mammals, including tumors and leukemia. Cancers include those malignant diseases which attack normal healthy cells. Leukemia encompasses those diseases which attack normal healthy blood cells and bone marrow which produce bone cells which are found in mammals.

As used herein, the "anti-cancer compounds" are the benzimidazoles, and their salts. The exact benzimidazoles are described in detail below. The preferred materials are the products sold under the names "thiabendazole®", "benomyl®" and "carbendazim®" by BASF and Hoechst, DuPont and MSD-AgVet.

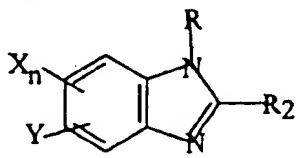
As used herein "viruses" includes viruses which infect animals or mammals, including humans. Viruses includes HIV, influenza, polio viruses, herpes, rhinoviruses, and the like.

As used herein "chemotherapeutic agents includes DNA-interactive Agents, Antimetabolites, Tubulin-Interactive Agents, Hormonal agents and others, such as Asparaginase or hydroxyurea.

As used herein "potentiators" are materials such as triprolidine and its cis-isomer and procodazole which are used in combination with the chemotherapeutic agents and benzimidazoles.

B. THE ANTI-CANCER COMPOUNDS

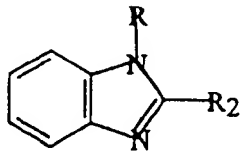
The anti-cancer compounds are benzimidazole derivatives which are known for their antifungal activities. They are systemic fungicides used to prevent and eradicate fungi. The compounds have the following structure:



wherein X is hydrogen, halogen, alkyl of less than 7 carbon atoms or alkoxy of less than 7 carbon atoms; n is a positive integer of less than 4; Y is hydrogen, chlorine, nitro, methyl or ethyl; and R

is hydrogen or an alkyl group having from 1 to 8 carbons, and R_2 is 4-thiazolyl, $NHCOOR_1$ wherein R_1 is aliphatic hydrocarbon of less than 7 carbon atoms, and preferably and alkyl group of less than 7 carbon atoms.

Preferably the compositions are:



wherein R is an alkyl of 1 through 8 carbon atoms and R_2 is selected from the group consisting of 4-thiazolyl, $NHCOOR_1$, wherein R_1 is methyl, ethyl or isopropyl and the non-toxic, pharmaceutically acceptable acid addition salts with both organic and inorganic acids.

The most preferred compounds are 2-(4-thiazolyl)benzimidazole, methyl -(butylcarbamoyl)-2-benzimidazolecarbamate and 2-methoxycarbonylamino-benzimidazole and the compounds wherein Y is chloro and X is hydrogen.

These compounds are prepared according to the method described in U.S. 3,738,995 issued to Adams et al, June 12, 1973. The thiazolyl derivatives are prepared according to the method described in Brown et al., J. Am. Chem. Soc., 83, 1764 (1961) and Grenda et al., J. Org. Chem., 30, 259 (1965).

It is believed that fungicides, particularly systemic fungicides, have the capability of reducing tumors or decreasing their growth significantly. Systemic fungicides have the ability to migrate through the plant or animal body. While this is a positive attribute, it is not an essential requirement of the effective compounds for treating viral infections, cancers or tumors.

C. CHEMOTHERAPEUTIC AGENTS

The chemotherapeutic agents are generally grouped as DNA-interactive Agents, Antimetabolites, Tubulin-Interactive Agents, Hormonal agents and others such as Asparaginase or hydroxyurea. Each of the groups of chemotherapeutic agents can be further divided by type of activity or compound. The chemotherapeutic agents used in combination with the anti-cancer agents or benzimidazoles of this invention include members of all of these groups. For a detailed discussion of the chemotherapeutic agents and their method of administration, see Dorr, et al, *Cancer Chemotherapy Handbook*, 2d edition, pages 15-34, Appleton & Lange (Connecticut, 1994) herein incorporated by reference.

DNA-Interactive Agents include the alkylating agents, e.g. Cisplatin, Cyclophosphamide, Altretamine; the DNA strand-breakage agents, such as Bleomycin; the intercalating topoisomerase II inhibitors, e.g., Dactinomycin and Doxorubicin); the nonintercalating topoisomerase II inhibitors such as, Etoposide and Teniposide; and the DNA minor groove binder Plicamydin.

The alkylating agents form covalent chemical adducts with cellular DNA, RNA, and protein molecules and with smaller amino acids, glutathione and similar chemicals. Generally, these alkylating agents react with a nucleophilic atom in a cellular constituent, such as an amino, carboxyl, phosphate, sulfhydryl group in nucleic acids, proteins, amino acids, or glutathione. The mechanism and the role of these alkylating agents in cancer therapy is not well understood. Typical alkylating agents include:

Nitrogen mustards, such as Chlorambucil, Cyclophosphamide, Isofamide, Mechlorethamine, Melphalan, Uracil mustard;

aziridines such as Thiotepa;

methanesulfonate esters such as Busulfan;

nitroso ureas, such as Carmustine, Lomustine, Streptozocin;

platinum complexes, such as Cisplatin, Carboplatin;

bioreductive alkylator, such as Mitomycin, and Procarbazine, Dacarbazine and Altretamine;

DNA strand breaking agents include Bleomycin;

DNA topoisomerase II inhibitors include the following:

Intercalators, such as Amsacrine, Dactinomycin, Daunorubicin, Doxorubicin, Idarubicin, and Mitoxantrone;

nonintercalators, such as Etoposide and Teniposide.

The DNA minor groove binder is Plicamycin.

The antimetabolites interfere with the production of nucleic acids by one or the other of two major mechanisms. Some of the drugs inhibit production of the deoxyribonucleoside triphosphates that are the immediate precursors for DNA synthesis, thus inhibiting DNA replication. Some of the compounds are sufficiently like purines or pyrimidines to be able to substitute for them in the anabolic nucleotide pathways. These analogs can then be substituted into the DNA and RNA instead of their normal counterparts. The antimetabolites useful herein include:

folate antagonists such as Methotrexate and trimetrexate

pyrimidine antagonists, such as Fluorouracil, Fluorodeoxyuridine, CB3717, Azacytidine, Cytarabine, and Floxuridine

purine antagonists include Mercaptopurine, 6-Thioguanine, Fludarabine, Pentostatin;

sugar modified analogs include Cyctrabine, Fludarabine;

ribonucleotide reductase inhibitors include hydroxyurea.

Tubulin Interactive agents act by binding to specific sites on tubulin, a protein that polymerizes to foam cellular microtubules. Microtubules are critial cell structure units. When the interactive agents bind on the protein, the cell can not form microtubules Tubulin Interactive agents include Vincristine and Vinblastine, both alkaloids and Paclitaxel.

Hormonal agents are also useful in the treatment of cancers and tumors. They are used in hormonally susceptible tumors and are usually derived from natural sources. These include:

estrogens, conjugated estrogens and Ethinyl Estradiol and Diethylstilbesterol, Chlorotrianisene and Idenestrol;

progestins such as Hydroxyprogesterone caproate, Medroxyprogesterone, and Megestrol;

androgens such as testosterone, testosterone propionate; fluoxymesterone, methyltestosterone;

Adrenal corticosteroids are derived from natural adrenal cortisol or hydrocortisone. They are used because of their anti inflammatory benefits as well as the ability of some to inhibit mitotic divisions and to halt DNA synthesis. These compounds include, Prednisone, Dexamethasone, Methylprednisolone, and Prednisolone.

Leutinizing hormone releasing hormone agents or gonadotropin-releasing hormone antagonists are used primarily the treatment of prostate cancer. These include leuprolide acetate and goserelin acetate. They prevent the biosynthesis of steroids in the testes.

Antihormonal antigens include:

antiestrogenic agents such as Tamosifen,

antiandrogen agents such as Flutamide ; and

antiadrenal agents such as Mitotane and Aminoglutethimide.

Hydroxyurea appears to act primarily through inhibition of the enzyme ribonucleotide reductase.

Asparagenase is an enzyme which converts asparagine to nonfunctional aspartic acid and thus blocks protein synthesis in the tumor.

D. POTENTIATORS

The "potentiators" can be any material which improves or increase the efficacy of the pharmaceutical composition and/or an immuno suppressor. One such potentiator is triprolidine and its cis-isomer which are used in combination with the chemotherapeutic agents and the benzimidazole. Triprolidine is described in US 5,114,951 (1992). Another potentiator is procodazole, 1H-Benzimidazole-2-propanoic acid; [ß-(2-benzimidazole) propionic acid; 2-(2-carboxyethyl) benzimidazole; propazol) Procodazole is a non-specific active immunoprotective agent against viral and bacterial infections and can be used with the compositions claimed herein. It is effective with the benzimidazoles alone in treating cancers, tumors, leukemia and viral infections or with the combined benzimidazoles and chemotherapeutic agents. Propionic acid and its salts and esters can also be used in combination with the pharmaceutical compositions claimed herein.

The potentiators also improve the efficacy of the benzimidazole compounds in treating viruses and other infections. They can be used in conjunction with these anti-cancer agents in a

safe and effective amount. These combinations can be administered to the patient or animal by oral, rectal, topical or parenteral administration.

Antioxidant vitamins such as ascorbic acid, beta-carotene, vitamin A and vitamin E can be administered with the compositions of this invention.

E. DOSAGE

Any suitable dosage may be given in the method of the invention. The type of compounds and the carriers and the amount will vary widely depending on the species of the warm blooded animal or human, body weight, and cancer, or tumor being treated. The range and ratio of the chemotherapeutic agent and anti cancer compound used will depend on the type of chemotherapeutic agent and the cancer being treated. Generally a dosage of between about 2 milligrams (mg) per kilogram (kg) of body weight and about 400 mg per kg of body weight is suitable. Preferably from 15 mg to about 150 mg/kg of body weight is used for the benzimidazoles. For the chemotherapeutic agents, a lower dosage may be appropriate, i.e., from about 0.5 mg/kg of body weight to about 400 mg/kg body weight. Generally, the dosage in man is lower than for small warm blooded mammals such as mice. A dosage unit may comprise a single compound or mixtures thereof with other compounds or other cancer inhibiting compounds. The dosage unit can also comprise diluents, extenders, carriers and the like. The unit may be in solid or gel form such as pills, tablets, capsules, liposomes and the like or in liquid form suitable for oral, rectal, topical, intravenous injection or parenteral administration or injection into or around the tumor or into or around the bone marrow.

F. DOSAGE DELIVERY FORMS

The anti-cancer compounds and chemotherapeutic agents are typically mixed with a pharmaceutically acceptable carrier. This carrier can be a solid or liquid or a liposome and the type is generally chosen based on the type of administration being used. The active agent can be coadministered in the form of a tablet or capsule, liposome, or as an agglomerated powder or in a liquid form. Examples of suitable solid carriers include lactose, sucrose, gelatin and agar. Capsule or tablets can be easily formulated and can be made easy to swallow or chew; other solid forms include granules, and bulk powders. Tablets may contain suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. Examples of suitable liquid dosage forms include solutions or suspensions in water, pharmaceutically acceptable fats and oils, alcohols or other organic solvents, including esters, emulsions, syrups or elixirs, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Such liquid dosage forms may contain, for example, suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, thickeners, and melting agents. Oral dosage forms optionally contain flav rants and coloring agents. Parenteral and intravenous forms would

also include minerals and other materials to make them compatible with the type of injection or delivery system chosen.

Specific examples of pharmaceutical acceptable carriers and excipients that may be used to formulate oral dosage forms of the present invention are described in US. Pat. No. 3,903,297 to Robert, issued Sept. 2, 1975. Techniques and compositions for making dosage forms useful in the present invention are described in the following references: 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, Editors, 1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition (1976).

G. METHOD OF TREATMENT

The method of treatment can be any suitable method which is effective in the treatment of the particular cancer or tumor type that is being treated. Treatment may be oral, rectal, topical, parenteral or intravenous administration or by injection into the tumor and the like. The method of applying an effective amount also varies depending on the tumor being treated. It is believed that parenteral treatment by intravenous, subcutaneous, or intramuscular application of the benzimodale compounds, formulated with an appropriate carrier, additional cancer inhibiting compound or compounds or diluent to facilitate application will be the preferred method of administering the compounds to warm blooded animals.

The method of treating viral infections may also be by oral, rectal, topical, parenteral or intravenous administration. The actual time and dosage will depend on the virus being treated and the desired blood levels.

These same systemic fungicides can be used alone or in combination with other fungicides along with the chemotherapeutic agents.

Other fungicides that can be used with these materials include 1H-1, 2, 4-triazole derivatives such as fluconazole, and propiconazole, and N-chlorophenythiocarbamates. Herbicides such as N-phosphonoglycine derivatives, e.g. glyphosate can also be used in combination with the benzimidazoles.

The following examples are illustrative and are not meant to be limiting to the invention.

Example I

Colon, Breast and Lung Tumor Cells Test

The following cell culture tests were performed to test the toxicity of the benzimidazole compounds on colon, breast and lung human tumor cells. The viability of the cells were tested by looking at MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide) reduction. MTT assay is a well known measure of cell viability.

The colon tumor cells (HT29 from American Type Culture Collection (ATCC)) and the breast cells (MX1 from cell lines from ATCC) were cultured in Eagle's Miminal Essential

Medium with 10% fetal bovine serum. The lung tumor cells (A549 from ATCC cell lines) were cultured in Ham's F12 medium with 10% fetal bovine serum.

The tumor cells were passaged and seeded into culture flasks at the desired cell densities. The culture medium was decanted and the cell sheets were washed twice with phosphate buffered saline (PBS). The cells were trypsinized and triturated prior to seeding the flasks. Unless otherwise indicated the cultures were incubated at 37° ± 1°C in a humidified atmosphere of 5± 1% carbon dioxide in air. The cultures were incubated until they were 50-80% confluent.

The cells were subcultured when the flasks were subconfluent. The medium was aspirated from the flasks and the cell sheets rinsed twice with PBS. Next, the Trypsin Solution was added to each flask to cover the cell sheet. The Trypsin Solution was removed after 30-60 seconds and the flasks were incubated at room temperature for two to six minutes. When 90% of the cells became dislodged, growth medium was added. The cells were removed by trituration and transferred to a sterile centrifuge tube. The concentration of cells in the suspension was determined, and an appropriate dilution was made to obtain a density of 5000 cells/ml. The cells were subcultured into the designated wells of the 96-well bioassay plates (200 microliter cell suspension per well). PBS was added to all the remaining wells to maintain humidity. The plates were then incubated overnight before test article treatment.

Each dose of test article was tested by treating quadruplicate wells of cultures with 100 microliter of each dilution. Those wells designated as solvent controls received an additional 100 microliter of methanol control; negative controls wells received an additional 100 microliters of treatment medium. PBS was added to the remaining wells not treated with test article or medium. The plates were then incubated for approximately 5 days.

At the end of the 5 day incubation, each dose group was examined microscopically to assess toxicity. A 0.5 mg/ml dilution of MTT was made in treatment medium, and the dilution was filtered through a 0,.45 micrometer filter to remove undissolved crystals. The medium was decanted from the wells of the bioassy plates. Immediately thereafter, 2000 microliter of the filtered MTT solution was added to all test wells except for the two untreated blank test wells. The two blank wells received 200 microliters of treatment medium. The plates were returned to the incubator for about 3 hours. After incubation, the MTT containing medium was decanted. Excess medium was added to each well and the plates were shaken at room temperature for about 2 hours.

The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices (Menlo Park, CA) VMax plate reader.

The mean OD_{550} of the solvent control wells and that of each test article dilution, and that of each of the blank wells and the positive control were calulated. The mean OD_{550} of the blank wells were subtracted from the mean of the solvent contrl wells, and test srticle wells, respectively to give teh corresponding mean OD_{550}.

$$\% \text{ of Control} = \frac{\text{corrected mean } OD_{550} \text{ of Test Article Dilution}}{\text{corrected mean of } OD_{550} \text{ of Solvent Control}} \times 100$$

Dose response curves were prepared as semi-log plots with % of control on the ordinate (linear) and the test article concentration on the abscissa (logarithmic). The EC_{50} was interpolated from the plots for each test article.

For the test articles administered in methanol, separate responses were prepared to correct for the methanol data.

Adriamycin was used as a positive control. In all cases, it was more toxic than any of the test materials by one or two logs. Adriamycin is one of the more potent agents in current use and one with significant side effects. The peak plasma concentration of other, quite effective chemotherapeutic agents may be 10 to 50 times higher than that of Adriamycin.

The EC_{50} is the concentration at which one half of the cells are killed.

Table 1

Test Material	EC-50 Result (ppm)					
	HT29	HT29	MX1	MX1	A549	A549
Benomyl	0.728	0.682	3.26	2.4	3.24	2.81
Carbendazin	0.320	0.506	0.752	0.822	1.52	1.42
Adriamycin	0.015	0.0020	0.0035	0.0093	0.065	0.10

In normal healthy cells, the following results were obtained. As is evident, the benomyl and carbendazim were much less toxic to normal heathly cells than adriamycin.

Table 2

Test Material	EC-50					
	Broncheal Cells		Kerotinovle Cells		Fibroblasts	
Benomyl	0.728	0.682	3.26	2.4	3.24	2.81
Carbendazin	0.320	0.506	0.752	0.822	1.52	1.42
Adriamycin	0.015	0.0020	0.0035	0.0093	0.065	0.10

In a related study using lung tumor cells (A-549) breast tumor cells (MCF-7) and colon tumor cells (HT-29), thiabendazol, a systemic fungicide, effectively killed these cells. Table 3 summarizes the results.

Table 3

Concentration (ppm)	Optical Density		
	A-549	MCF-7	HT-29
0-Control	0.600	0.245	0.398
173	0.007	0.007	0.005
35	0.411	0.025	0.011
17.3	0.851	0.258	0.204
3.46	1.12	0.466	0.713
0.87	1.32	0.507	0.852

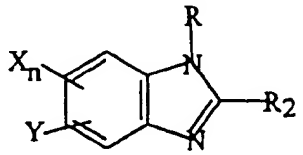
These experiments show that these compositions are effective in killing tumor cells.

Example II

Mice are randomly selected and divided into groups for treatment. Five groups are infected with leukemia. The diseased animals are dosed for five days, off two days and then dosed for another five days and then three days off, then dosed for five days and off for two days. This dosing on and off in an irregualr pattern was not an ideal regimien, but the results do show a positive benefit for the Carbendazim™. One group of mice was treated with Cytoxan™, 2-[bis(2-chloroethyl)-amino-1-oxo-2-aza-5-oxophosphoridin, a control was dosed with canola oil and three groups were treated with various levels of Carbendazim™, methyl -(butylcarbamoyl)-2-benzimidazole-carbamate. A control with no treatment was also used. The Carbendazim™ was dosed at three levels 4000 mg/kg, 2500 mg/kg and 1000 mg/kg. The Cytoxan™ was dosed at 125 mg/kg. After 8 days, the no treatment group had lost 1 mouse, by day 10, 8 mice were dead and at day 11 all ten mice were dead. The mice in the Cytoxan™ group survived more than 21 days. The higher dose Carbendazim™ group had one mouse die on day 14, two died on days 15,16 and 17 and one each died on days 20, 21, and 22. The mean number of days for this group is 17.3. The intermediate dosage group had 2 mice die on day 14, 4 on day 15, 1 on day 16, 2 on day 19 and 1 on day 21. The mean number of days for this group is 16.50. The lowest dosage group had 2 mice die on day 12, 13, 14, and 15; and 1 died on each of days 16 and 17. The mean number of days for this group is 14.1.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for treating cancers, tumors or viral infections comprising a safe and effective amount of:



wherein X is hydrogen, halogen, alkyl of less than 7 carbon atoms or alkoxy of less than 7 carbon atoms; n is a positive integer of less than 4; Y is hydrogen, chlorine, nitro, methyl or ethyl; and R is hydrogen or an alkyl group having from 1 to 8 carbon atoms, and R_2 is 4-thiazolyl or $NHCOOR_1$ wherein R_1 is aliphatic hydrocarbon of less than 7 carbon atoms or the pharmaceutically acceptable inorganic or acid addition salts thereof.

2. A pharmaceutical composition according to Claim 1 further comprising a safe and effective amount of a chemotherapuetic agent.

3. A pharmaceutical composition according to Claim 1 further comprising a safe and effective amount of a potentiator.

4. A pharmaceutical composition wherein R is hydrogen or an alkyl having from 1 to 8 carbon atoms and R_2 is selected from the group consisting of 4-thiazolyl, $NHCOOR_1$, wherein R_1 is methyl, ethyl or isopropyl and the pharmaceutically acceptable organic or inorganic acid addition salts thereof.

5. A pharmaceutical composition according to Claim 1,2,3 or 4 wherein said benzimidazole is selected from the group consisting of 2-(4-thiazolyl)benzimidazole, methyl -(butylcarbamoyl)-2-benzimidazolecarbamate and 2-methoxycarbonylamino-benzimidazole.

6. A pharmaceutical composition according to Claim 5 for inhibiting the growth of tumors and wherein said chemotherapeutic agent is selected from the group consisting of DNA-interactive Agents, Antimetabolites, Tubulin-Interactive Agents, Hormonal agents, Asparaginase or hydroxyurea, Asparaginase,

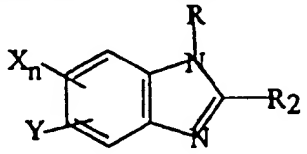
hydroxyurea, Cisplatin, Cyclophosphamide, Altretamine; Bleomycin, Dactinomycin, Doxorubicin, Etoposide, Teniposde, and Plcamydin, Methotrexate, Fluorouracil, Fluorodeoxyuridine, CB3717, Azacytidine, Cytarabine, and Floxuridine, Mercaptopurine, 6-Thioguanine, Fludarabine, Pentostatin, Cyctrabine, and Fludarabine.

7. A pharmaceutical composition according to Claim 6 wherein said pharmaceutical acceptable acid addition salts are selected from the group consisting of chlorides, bromides, sulfates, nitrates, phosphates, sulfonates, formates, tartrates, maleates, malates, citrates, benzoates, salicylates, ascorbates and mixtures thereof and wherein Y is chloro.

8. A method of treating cancer in warm blooded mammals comprising administering from about 2 mg/kg body weight to about 400 mg/kg of a pharmaceutical composition according to claims 1,2,3,4,5,6 or 7.

9. A method according to Claim 8 wherein said benzimidazole is administered orally or enterically, intravenously, parenterally, or by injection into the tumor.

10. A unit dosage composition for treating cancer and viral infections in animals or humans comprising a benzimiadozole of the formula:



wherein X is hydrogen, halogen, alkyl of less than 7 carbon atoms or alkoxy of less than 7 carbon atoms; n is a positive integer of less than 4; Y is hydrogen, chlorine, nitro, methyl or ethyl; and R is hydrogen or an alkyl group having from 1 to 8 carbon atoms, and R_2 is 4-thiazolyl or $NHCOOR_1$ wherein R_1 is aliphatic hydrocarbon of less than 7 carbon atoms and pharmaceutically acceptable salts thereof.

11. A unit dosage composition according to claim 10 further comprising a chemotherapeutic agent.

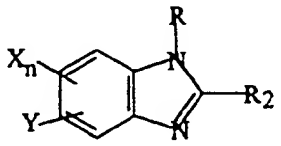
12. A unit dosage composition according to claim 10 or 11 further comprising a potentiator.

13. A unit dosage composition according to Claim 21 wherein said benzimidazole is selected from the group consisting of 2-(4-thiazolyl)benzimidazole, methyl -(butylcarbamoyl)-2-benzimidazolecarbamate and 2-methoxycarbonylamino-benzimidazole and the pharmaceutically acceptable salts thereof.

14. A unit dosage composition according to Claim 10,11, or 12 wherein said carrier selected from the group consisting of lactose, sucrose, gelatin and agar, aqueous solutions, emulsions, suspension solutions, and suspensions reconstituted from non-effervescent and effervescent preparations.

15. A unit dosage composition according to Claim 13 wherein said dosage composition contains a member selected from the group consisting of suspending agents, diluents, sweeteners, flavorants, colorants, preservatives, emulsifying agents and coloring agents, and mixtures thereof.

16. A method of treating viral infections comprising administering a safe and effective amount of:



wherein X is hydrogen, halogen, alkyl of less than 7 carbon atoms or alkoxy of less than 7 carbon atoms; n is a positive integer of less than 4; Y is hydrogen, chlorine, nitro, methyl or ethyl; and R is hydrogen or an alkyl group having from 1 to 8 carbon atoms, and R_2 is 4-thiazolyl or $NHCOOR_1$ wherein R_1 is aliphatic hydrocarbon of less than 7 carbon atoms and the pharmaceutically acceptable organic or inorganic addition salts thereof.

16. A method of treating malignant tumors comprising administering a safe and effective amount of a fungicide.

17. A method of inhibiting the growth of tumors comprising administering a safe and effective amount of a fungicide.

INTERNATIONAL SEARCH REPORT

In' tional Application No
PCT/US 96/04955

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/425 A61K31/415

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER TREAT.REP., vol. 62, no. 11, 1978, pages 1955-62, XP000579564 LUNDY ET AL.: "Immunomodulation with thiabendazole: a review of immunologic properties and efficacy in combined modality cancer therapy" see page 1958, right-hand column see page 1959, left-hand column, line 3 - right-hand column, line 4 see page 1960, left-hand column, line 3 - line 6 --- -/--	1-15,17, 18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
23 August 1996	05.09.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Gerli, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/04955

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DISS.ABSTR.INT.(SCI.), vol. 39, no. 11, 1979, pages 5315-5316, XP000579587 LOVETT: "Immunomodulating effects of thiabendazole: immunotherapeutic efficacy in the treatment of a murine fibrosarcoma" see page 5316, left-hand column, line 8 - last line	1-15,17, 18
X	SURG.FORUM, vol. 27, no. 62, 1976, pages 132-4, XP002011468 LUNDY ET AL.: "Thiabendazole: a new immunopotentiator effective in therapy of murine fibrosarcoma" see the whole document	1-15,17, 18
Y	TOXICOLOGY, vol. 94, no. 1-3, 1994, pages 173-85, XP000579566 MARINOVICH ET AL.: "Mixtures of benomyl, pirimphos-methyl, dimethoate, diazinon, and azinphos-methyl affect the protein synthesis in HL-60 cells differently" see page 175, paragraph 2 see page 176, paragraph 3	1-15,17, 18
Y	US,A,5 114 951 (KING) 19 May 1992 cited in the application see the whole document	1-15,17, 18

1

INTERNATIONAL SEARCH REPORT

Int- -ational application No.
PCT/US96/04955

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 8, 9, 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
For the purpose of the search report, claim 16 and 17 on page 16 have been renumbered as 17 and 18.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/US 96/04955

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5114951	19-05-92	NONE	